marsh mosquito, *A. cantator* (Coq.). Control recommendations are briefly outlined.

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LABORATORY STUDIES ON THE HATCHING OF MARSH-MOSQUITO EGGS

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As a part of a project on the biology of salt-marsh mosquitoes in Florida, extensive laboratory studies were conducted from 1939 to 1941 to develop a method of flooding that would insure a complete hatch of mosquito eggs on soil samples collected from salt marshes for population studies (Bradley and Travis 1942, Travis and Bradley 1943). This paper reports preliminary laboratory tests with various hatching media and the effect of desiccation on the viability of the eggs. Data on the flooding of soil samples collected for egg-population studies are also summarized.

MATERIALS AND METHODS. Eggs used in the tests were laid in the laboratory by the salt-marsh mosquitoes *Aedes taeniorhynchus* (Wied.) and *sollicitans* (Wlkr.), and by one fresh-marsh species, *Psorophora confinnis* (L.-Arr.). The mosquitoes were collected in the field either from a baffle trap baited with a rabbit or from the arms of the laboratory workers.

All the mosquitoes that were engorged were transferred to lantern-globe cages for oviposition. The top of the globe was covered with gauze, on which were placed honey and raisins for food. The bottom was placed in a half petri dish lined with wet paper toweling. Each morning the eggs were washed from the toweling and concentrated by filtration. Consequently each lot of eggs could have been on a wet surface for as long as 24 hours before being used in a test.

Except for one test, in which the eggs were not dried, the eggs were alternately dried and then flooded with various media until there was no further hatching. They were placed in small glass vials or on soil samples in 3-inch evaporating dishes for flooding. At the end of each test the unhatched eggs were dissected to determine whether any were still viable. Unless otherwise stated, the unhatched eggs were found to be nonviable when dissected.

TESTS WITH VARIOUS HATCHING MEDIA. The basic hatching media were distilled, rain, tap, and sea water. To these waters were added solutions of asparagine, and infusions of leaves, coke or charcoal, and soil from salt marshes. The leaf infusions were prepared by triturating 1 to 100 grams of green leaves of saltwort, glasswort, or black or white mangrove in 1 liter of water. The asparagine solutions, 2 parts by weight of asparagine to 1 part of sodium biphosphate, were prepared in

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different concentrations. The coke and charcoal were added at the rate of 1 gram to 10 ml., and the soil was added at 0.5 to 1 gram per liter of water.

Eggs to be used in these tests were left on filter paper and placed on sand kept wet with rain water for 6 to 10 days to insure full development of the embryos. The eggs were then stored in glass vials until they were to be used in a test.

Untreated water.—Preliminary tests were made with eggs of *taeniorhynchus, sollicitans,* and *confinnis* that had been wet and dry for various periods of time. Most of these eggs were repeatedly flooded in clean vials with untreated tap water.

Table 1 shows that similar results were obtained with *taeniorhynchus* and *confinnis* eggs, the greatest hatch occurring with the third flooding. Most of the *sollicitans* eggs hatched with the first flooding. More floodings were necessary to hatch eggs of *confinnis* than of either of the other species.

Two viable eggs of *sollicitans* and 11 of *confinnis* hatched in subsequent floodings with asparagine.

Asparagine solutions.—Asparagine at concentrations ranging from 0.001 to 20 percent in distilled water caused 97 to 100 percent hatch of *taeniorhynchus* eggs that had been stored dry for 4 to 8 days (table 2). Little or no difference in the effectiveness of different concentrations of asparagine was shown in tests with lots of eggs that had been stored 4 to 46 days, but with eggs stored for 73 to 83 days fewer eggs hatched in the 0.001 percent solution than in solutions containing more asparagine. There was a marked difference in the percentage hatch of eggs of different ages, the hatch being considerably less for the eggs 73 to 83 days old than for those 26 to 46 days old.

Eggs of *sollicitans* hatched equally well in tap, rain, and distilled water to which asparagine had been added (table 3), but the hatch was lower when this chemical was added to sea water. The lowest hatch with sea water occurred with the highest concentration (2 percent) of asparagine, but there was little difference between hatches at other concentrations. With the other waters fewer eggs hatched with 0.0001 percent of asparagine than with higher concentrations, which gave about equal hatches. The hatch of eggs treated with the 0.0001 percent of asparagine was only slightly different from that of eggs flooded with untreated water. There was no difference in the number of eggs of the same age hatching when flooded with the four test waters containing no asparagine.

When data on the percentages of eggs that hatched with each flooding of asparagine solutions were assembled (not given in the tables), it was found that all eggs of both *Aedes* species hatched with two floodings of asparagine-treated water. The first flooding caused 81 percent of the *sollicitans* eggs and 90 percent of the *taeniorhynchus* eggs to hatch. Most of the eggs that failed to hatch were in solutions containing less than 0.01 percent of asparagine.

Leaf infusions.—Infusions prepared with

TABLE 1.—Percentage of eggs hatching with each flooding of untreated water. (120 10-egg samples for all species and 20 additional 50-egg samples for *Aedes taeniorhynchus*)

Flooding	*Aedes taeniorhynchus*	*Aedes sollicitans*	*Psorophora confinnis*
First	0.3	63.8	0.3
Second	12.6	29.7	13.2
Third	84.3	6.5	71.4
Fourth	2.5	0	7.9
Fifth	.3	0	1.0
Sixth	0	—	4.5
Seventh	0	—	1.7

TABLE 2.—Percentage of *Aedes taeniorhynchus* eggs hatching in distilled water containing asparagine after being stored dry for different periods. (10 to 30 10-egg samples for each concentration and each storage period.)

Concentration of Asparagine (percent)	4–8 Days	26–46 Days	73–83 Days
20	100	70	24
5	100	70	50
0.1	100	70	45
.01	97	—	35
.001	100	—	10
Untreated water (check)	—	85	58

TABLE 3.—Percentage of *Aedes sollicitans* eggs hatching in asparagine solutions made with different kinds of water

Concentration of Asparagine (percent)	Stored Dry for 133–142 Days; (110 10-Egg Samples)				Stored Dry for 4–42 Days; (45 10-Egg Samples)
	Tap	Sea	Rain	Distilled	Distilled
2	88	2	92	—	86
0.5	88	26	88	86	98
.01	73	32	81	90	100
.001	69	28	82	92	98
.0001	58	42	46	58	84
Untreated water (check)	50	51	52	54	92

black mangrove leaves in tap, distilled, and rain water each gave about the same hatch of *sollicitans* eggs, but the hatches were somewhat smaller than with asparagine solutions. The infusions made with sea water showed almost negligible hatches (table 4). Because the hatching of *taeniorhynchus* paralleled that of *sollicitans*, data on this species are not presented.

When the data on all floodings with leaf infusions were assembled (not shown in tables), it was found that only two of several thousand eggs of *taeniorhynchus* and none of *sollicitans* failed to hatch with two floodings. The first flooding caused 91 percent of the *taeniorhynchus* eggs to hatch. Most of the eggs that failed to hatch were in infusions containing less than 0.01 percent of leaves. Results with leaf infusions of saltwort, glasswort, and white mangrove were almost identical with the data presented for black mangrove.

Coke or charcoal and soil infusions.—Several tests were made in which coke or charcoal was added to the flooding water. In each test soil was added 24 hours after the eggs had been flooded. Water containing softwood charcoal caused no hatching of either *taeniorhynchus* or *sollicitans* eggs until soil was added. The hatches with powdered coke were 25 percent for *taeniorhynchus* and 10 per cent for *sollicitans,* and with hardwood charcoal, 5 percent and 25 percent respectively. Untreated water caused 15 percent of the *taeniorhynchus* and 10 percent of the *sollicitans* eggs to hatch. All remaining eggs hatched when soil was added.

Asparagine solutions with and without soil.—A series of preliminary tests was made in which eggs of *taeniorhynchus* were flooded with asparagine solutions, with and without soil. The results, given in table 5, show no increase in hatch when soil was added to the sea-water solutions and a slight decrease in hatch when added to rain-water solutions. All the hatches were high with one flooding, however,

TABLE 4.—Percentage of *Aedes sollicitans* eggs hatching in infusions of black mangrove leaves made with different kinds of water. (Eggs stored dry 135–147 days; 15 10-egg samples for tap water and 10 for the other waters.)

Leaves per Liter of Water (grams)	Tap	Distilled	Rain	Sea
100	59	52	64	—
5	82	68	36	0
1	75	58	50	8
0.5	72	82	60	2
.1	40	76	48	20
Untreated water (check)	54	53	54	55

TABLE 5.—Percentage of *Aedes taeniorhynchus* eggs hatching in asparagine solutions, with and without soil, made from rain and sea water. (Eggs stored 10–20 days; 5 10-egg samples.)

Concentration of Asparagine (percent)	Rain Water		Sea Water	
	First Flooding	Second Flooding	First Flooding	Second Flooding
0.1	98	0	87	2
.1 plus soil	90	1	87	5
.05	98	2	95	1
.05 plus soil	79	17	97	2
.01	100	0	97	1
.01 plus soil	96	3	96	1
Untreated water (check)	2	81	0	83
Filtrate of soil and untreated water	98	0	94	0

TABLE 6.—Percentage of *Aedes taeniorhynchus* eggs hatching after being placed on soil samples and flooded three times in the laboratory with rain or sea water, alone or with 0.01 percent of asparagine. (30 to 36 10-egg samples in each treatment.)

Hatching Medium	Eggs Air-Dry for—	
	10 Days	63–70 Days
Rain water	53	18
Rain water plus asparagine	71 [1]	14
Sea water	62	11
Sea water plus asparagine	52	9

[1] One viable egg after first flooding.

TABLE 7.—Number of salt-marsh *Aedes* larvae on soil samples and percentage of eggs hatching at each flooding with rain water

Number of Soil Samples	Total Number of Larvae	Percent Hatching at Indicated Flooding				
		First	Second	Third	Fourth	Fifth
1,631	2,559	99	1	—	—	—
3,749	8,124	99	1	+	—	—
474	1,548	92	6.1	1.7	0	—
35	475	90	2.7	5.9	1.7	0

and complete after the second. A filtrate of soil and untreated water caused practically complete hatch with one flooding.

In another series of tests, eggs were placed on soil samples and flooded with rain or sea water, with or without asparagine. As shown in table 6, only one viable egg remained after the first flooding.

FLOODING OF SOIL SAMPLES. Data from soil-sample studies are summarized here to show how many of the eggs hatched with each flooding of rain water. Only rarely were *sollicitans* mosquitoes taken from samples, but *taeniorhynchus* constituted well over 90 percent of the total hatch. Some of the soil samples were flooded from two to five times with a drying period between each flooding, but several series of samples were discarded when no hatching occurred. In most series, however, all samples were flooded three times even though there was no hatching at the second flooding.

Table 7 shows that 90 percent or more of the eggs hatched with the first flooding. However, on the average 98 percent of the eggs that hatched did so with the first flooding.

EFFECT OF DESICCATION ON VIABILITY OF EGGS. Eggs wet and dry for various periods.—Two series of tests were made in which eggs of the three species were subjected to various degrees of desiccation.

In series 1 eggs of *taeniorhynchus* and *confinnis* were placed on squares of wet blotting paper. Half of these squares were placed in a dish to dry, and each day for 10 days one lot of these eggs for each species was placed on wet sand. The other squares were kept on wet sand, but each day for 10 days one lot of eggs for each species was removed from the wet sand and allowed to dry. At the end of the 10-day period all eggs were alternately dried and flooded with one of the four hatching media. Fifty eggs were used for each treatment and the tests were not replicated.

In series 2 *taeniorhynchus* and *sollicitans* eggs were placed on squares of dry blotting paper. These eggs dried immediately, whereas in series 1 the rapidity with which the wet squares became air-dry depended upon the evaporation rate. Six replications with 10 eggs in each treatment were used for each species.

Eggs of the three species were also stored under air-dry and humid conditions. The vials of eggs stored under dry conditions were placed on shelves in the laboratory. The vials of eggs stored under humid conditions were sealed in a large fruit jar with water on the bottom.

Of the eggs on wet blotting paper that were dried slowly, those of *taeniorhynchus* failed to hatch after 3 or more days of drying and those of *confinnis* failed to hatch after 5 or more days (table 8). The exceptions are as follows: One egg of *taeniorhynchus* hatched in the sample dried for 10 days and one egg of *confinnis* hatched in each of the samples dried for 6 or 7 days. Subsequent tests indicated that these unusual records were most likely due to eggs that had stuck to the bottom of the lantern globes and were not removed on the day of oviposition. Thus they had remained wet, and were inadvertently removed with eggs that were recently laid; therefore, they could have been several days old. This chance of error was corrected in the second series of tests, in which no eggs of *taeniorhynchus* or *sollicitans* hatched after even 1 day of drying.

When the eggs of *taeniorhynchus* and *sollicitans* were kept wet for a variable number of days before being dried, only one *taeniorhynchus* (series 1) egg hatched when kept wet only 1 day prior to drying. Many eggs of both species were viable after being wet only 2 days, and 3 days seemed necessary for the embryo to mature properly. Eggs of *sollicitans* seemed less damaged by early drying than did those of *taeniorhynchus*. The eggs of *confinnis* were more resistant to damage from drying than eggs of the salt-marsh species.

Some miscellaneous observations on the effect of age and desiccation on the viability of *taeniorhynchus* and *sollicitans* eggs are summarized in table 9. The data

have been assembled from the hatching-media tests, and are therefore somewhat fragmentary. Since they show rather definite trends, they might be of interest to others who are studying the bionomics of mosquito eggs.

Although only a few of the data in table 9 can be compared, the *taeniorhynchus* eggs appeared to be affected more rapidly by aging under air-dry conditions than did those of *sollicitans*. When eggs of both species were stored in a humidity chamber, they were viable much longer than when kept under air-dry conditions, and again the *taeniorhynchus* eggs were more affected by age.

Eggs of *confinnis* were strikingly more resistant to aging than the other two species kept in dry storage. Data not included in the table showed that 90 percent hatched after 212 days and 80 percent after 240 days of dry storage. No eggs hatched after 329 days of dry storage.

Eggs not dried.—An additional experiment was made to determine whether eggs of salt-marsh *Aedes* would hatch under laboratory conditions without being dried. One lot of *taeniorhynchus* and two lots of *sollicitans* eggs were taken from the oviposition cages, and several hundred of each placed immediately in vials, 1 by 3 inches, which were then filled with distilled, rain, tap, or sea water. All the eggs sank to the bottom of the vials immediately and remained there during the entire experiment. The water containing the *taeniorhynchus* eggs and one lot of the *sollicitans* eggs was stirred periodically. The vials were refilled twice, and samples of the eggs were removed at intervals to determine the percentage that had hatched.

Between the fourth and eighth days after oviposition, a few eggs of both species hatched in all but one of the vials, although the vials had been undisturbed and the eggs had not been dried after the

TABLE 8.—Percentage of mosquito eggs viable after being kept wet and dry for different periods while embryos were forming

Treatment (days)		Series 1		Series 2	
		Aedes taeniorhynchus	*Psorophora confinnis* [1]	*Aedes taeniorhynchus*	*Aedes sollicitans*
Dry	Wet	Eggs Dry During Initial Treatment			
1	9	22	30 (3)	0	0
2	8	12	18	0	0
3	7	6	16	0	0
4	6	0	18	0	0
5	5	0	10	0	0
6	4	0	2	0	0
7	3	0	2	0	0
8	2	0	0	0	0
9	1	0	0	0	0
10	0	2	0	0	0
Wet	Dry	Eggs Wet During Initial Treatment			
1	9	2	56 (2)	0	0
2	8	16	90 (1)	35	76
3	7	50	78	75	93
4	6	64	40 (1)	87	83
5	5	86	32	87	79
6	4	82	46	72	81
7	3	86	38	85	81
8	2	82	34	93	83
9	1	74	42	87	79
10	0	64	68 (4)	70	83

[1] Numbers in parentheses indicate the eggs that did not hatch and were still viable at the end of the experiment.

TABLE 9.—Effect of age and desiccation on the hatching of marsh-mosquito eggs after the embryos are formed. (10-egg samples)

Aedes taeniorhynchus			*Aedes sollicitans*		
Number of Samples	Days Dry	Percent of Eggs Hatching	Number of Samples	Days Dry	Percent of Eggs Hatching
		Stored Under Air-Dry Conditions			
15	4	99	15	4	95
15	6	99	15	6	99
15	8	87	24	16	95
220	10	95	15	21	97
8	26	74	16	22	93
36	45	74	15	36	95
10	46	62	15	42	88
32	73	49	—	—	—
30	83	31	60	133	63
5	196	0	55	135	76
5	203	0	60	136	76
5	205	0	60	137	73
5	207	0	60	138	43
5	284	0	160	142	52
—	—	—	130	147	45
—	—	—	85	159	12
—	—	—	8	171	8
—	—	—	5	198	4
—	—	—	5	199	0
—	—	—	5	204	2
—	—	—	5	207	0
—	—	—	5	237	0
—	—	—	5	243	0
		Stored in Humidity Chamber			
2	196	85	4	169	98
2	204	55	2	196	75
2	345	0	2	204	85
—	—	—	2	345	0

embryos were formed. The only vial in which no eggs hatched was the one containing *taeniorhynchus* and sea water. The next hatchings took place when the water and eggs in the vials were stirred on the 24th day after oviposition when 6 *sollicitans* eggs hatched in the vial flooded with sea water, and on the 142nd day when 4 eggs hatched in the vial flooded with tap water. No other eggs hatched despite additional stirrings until the 210th day, when the vials were refilled with fresh water. Many eggs hatched in all but the one vial containing *taeniorhynchus* eggs covered with sea water. On the 73rd day a sample of eggs was withdrawn with a pipette from each vial to determine how many had hatched. For *taeniorhynchus* the hatch in the distilled, rain, tap, and sea water was 4, 2, 42, and 0, respectively, and for *sollicitans* it was 21, 20, 62, and 17. When water was added on the 327th day, no eggs hatched, and when the eggs were dissected on the 336th day, no viable eggs remained.

The second lot of *sollicitans* eggs was submerged in rain water within 24 hours after oviposition. By the eighth day 8 percent of these eggs had hatched, although the vials had been undisturbed and the eggs had not been dried. The remaining eggs were then divided equally among vials of distilled, rain, tap, and sea water. There was additional hatching after the transfer of the eggs between the 8th and 11th day in all vials but the one containing sea water while the vials were undisturbed, and also when all the vials were refilled

with fresh water on the 19th and 68th days. The addition of fresh water on the 127th day caused hatching in only the rain water. In addition to the hatching in this second lot of eggs, some eggs hatched in all vials when they were stirred on the 51st day; but stirring on the 66th day caused hatching in only the distilled water. On the 123rd day samples were withdrawn to determine how many eggs had hatched. The percentage hatching in the distilled, rain, tap, and sea water was 25, 30, 23, and 22, respectively. On the 192nd day stirring caused some hatch in the rain and distilled water, but not in the tap or sea water. No eggs hatched when water was added to the vials on the 261st day, and there were no viable eggs on the 386th day.

DISCUSSION. A number of workers have studied hatching stimuli for mosquito eggs. These studies, as summarized by Bates (1949), record observations similar to those reported in this paper. Several other workers have made observations on the hatching of mosquito eggs present in soil or plant debris (Dunn 1926, Connell 1940, Filsinger 1940, Bradley and Travis 1942, Travis and Bradley 1943, Horsfall 1949, Wilkins and Breland 1949, Bodman and Gannon 1950, and Buxton and Breland 1952). In most of their work the eggs were hatched by flooding the soil and debris with untreated water or with plant infusions.

Most of the early workers associated hatching stimuli with organic materials. Gjullin *et al.* (1939 and 1941) list several organic chemicals that can cause a reduction of the dissolved oxygen in the flooding media and stimulate hatching. Recently (Abdel-Malek 1948) plant hormones have been shown to stimulate the hatching of *Aedes* eggs.

Regardless of the nature of the various compounds that may be found to be associated with egg-hatching stimuli, it is obvious from the work in this paper and that of others that adequate egg-hatching stimuli are present or will develop quickly in soil and plant debris. It is also shown that eggs of different marsh mosquitoes differ in their resistance to desiccation. The resistance to desiccation of the three test species was in the following order: *confinnis, sollicitans,* and *taeniorhynchus*. It is likely that this order is correlated with the type of environment in which these eggs are normally laid. *P. confinnis* eggs are laid on fresh-water marshes, and such marshes may become much drier than the salt marshes. It is also to be noted that more floodings were necessary to hatch eggs of *confinnis* than of either of the salt-marsh species.

Some of the results obtained in this study cannot now be explained. For instance, why should fewer *sollicitans* eggs be viable when flooded with untreated tap, rain, or distilled water than with those waters treated with asparagine solutions, as summarized in table 3. It should be pointed out again that the hatches as given in the table represent all the viable eggs as confirmed by dissections at the end of the tests. The data seem to show that the viability of eggs not hatching with the first flooding may have been altered by this flooding. Another puzzling point is that the viability of eggs stored for long periods of time was apparently damaged by sea water to which leaf infusions or asparagine solutions were added (tables 3, 4, and 6), whereas eggs stored for shorter periods (tables 5 and 6) seemed to hatch as well in sea water as in rain water in the presence of soil or asparagine. Furthermore, when kept submerged in sea water, the eggs of *taeniorhynchus* were rendered nonviable. This observation is difficult to explain, as *taeniorhynchus* eggs occur on marshes that are periodically flooded with full-strength sea water.

The data presented in this paper are incomplete, as they were not obtained to solve a research problem on hatching. They were obtained in connection with studies to find a means of getting a hatch on soil samples complete enough to use the data in population studies. It is hoped that these preliminary observations will stimulate other workers to make a detailed

study of the biology of marsh-mosquito eggs.

SUMMARY. Laboratory studies were undertaken to develop a method of flooding that would insure a complete hatch of mosquito eggs on soil samples collected from salt marshes for population studies.

When eggs of *Aedes taeniorhynchus* (Wied.), *sollicitans* (Wlkr.), and *Psorophora confinnis* (L.-Arr.) were flooded repeatedly with untreated water in clean vials in the laboratory, they continued to hatch through the fifth, third, and seventh floodings, respectively. The greatest percentage of *taeniorhynchus* and *confinnis* eggs hatched with the third flooding and of *sollicitans* with the first.

The addition of asparagine-sodium biphosphate solutions or infusions made with the leaves of marsh plants or soil to rain, tap, distilled, or sea water caused nearly all the *Aedes* eggs to hatch with the first flooding. The fewest eggs hatched in the treated sea water than in the other waters.

Nearly all the *taeniorhynchus* eggs placed on marsh-soil samples hatched with the first flooding. Fewer eggs hatched in the treated sea water than in the other waters. When eggs of *taeniorhynchus* were placed on marsh-soil samples, nearly all hatched with the first flooding of rain or sea water, with or without asparagine.

The hatching data from soil samples collected from salt marshes and flooded with rain water to determine egg populations showed that more than 90 percent of the *Aedes* eggs hatched with one flooding. A small amount of hatching can be expected with at least four floodings.

Eggs of *taeniorhynchus* and *sollicitans* submerged in rain, tap, or distilled water within 24 hours after being laid hatched without an interval of drying. Some hatching occurred between the 4th to 8th days and thereafter up to the 210th day when fresh water was added or when the eggs were stirred in the containers. Eggs of *sollicitans* hatched in a like manner in sea water, but no *taeniorhynchus* eggs hatched when kept in sea water.

When the eggs of the three test species were kept wet and dry for a variable number of days, many of them were viable after being kept wet for only 2 days, but 3 days seemed necessary for the embryos to properly mature. Eggs of *sollicitans* were less damaged by drying within the first 3 days than those of *taeniorhynchus*, but *confinnis* eggs were the most resistant to damage from drying. The same relationship held for eggs stored in air-dry glass vials.

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